submitted herewith a Revised Sequence Listing which includes all of the sequences present in the specification. No new matter has been added.

Applicants note that the Attorney's Docket Number of this application has changed to D0590/7003. Please use this docket number for future correspondence.

Respectfully submitted,

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Attorney Docket No.: D0590/7003
Dated: January 4, 2001
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Paragraphs of the Specification as Amended

At page 15, please replace the following paragraph:

Any vector containing a T7 promoter may be used, and which contains a multiple cloning site (there are many commercially available). Primers containing the complementary strand, both with the appropriate ends are designed. These primers can be hybridized, and if well designed, cloned in the vector of choice. The minimal sequence for a T7 promoter is TAATACGACTCACTATAGGGCGA (SEQ ID NO: 12). Although any vector can be used for the construction of a T7 expression vector there follows an example of how to achieve this with the vector pGEM-3zf (-).

At page 15, please replace the following paragraph:

Sequences of oGN1 and oGN2 are:

- oGN1: AGC TGT AAT ACG ACT CAC TAT AGG GCG AGA AGC TT (SEQ ID NO:13)
- oGN2: TCG AAA GCT TCT CGC ATA ATA GTG AGT CGT ATT AC (SEQ ID NO:14)

At page 27, please replace the following paragraph:

The resulting PCR product is digested with NheI and NcoI, as is the vector in which we want to clone, being the Fire vector pPD49.78. The resulting vector is pGN100 illustrated in Figure 2 oGN3: CAT GGC AGG ATG AAC ACG ATT AAC ATC GC (SEQ ID NO:15); oGN4: ATG GCC CCA TGG TTA CGG GAA CGC GAA GTC CG (SEQ ID NO:16) pGN100 is included.

At page 30-31, please replace the following paragraph:

The T7 polymerase coding sequence was PCT amplified from_λ CE6 (Novagen, Madison, USA) using the primers oGN26 (ATGGAATTCTTACGCGAACGCGAAGTCCG; SEQ ID NO:17) and oGN46 (CTCACCGGTAATGAACACGATTAACATCGC; SEQ ID NO:18), using standard procedures (PCT, [A practical] A practical approach, 1993, Ed. J. McPherson, et al, IRL Press). The resulting DNA fragment encoding for the T7 RNA polymerase was digested with AgeI and EcoRI and inserted into the Fire vector pPD97.82

digested with AgeI and EcoRI. The resulting construct encodes for an open reading frame of T7 RNA polymerase in fusion with the SV40 large T antigen nuclear localization signal (NLS) with amino acid sequence MTAPKKKRKVPV (SEQ ID NO:19). This nuclear localization signal sequence is required to translocate the T7 RNA polymerase from the cytoplasm to the nucleus, where it is able to bind to its specific promoters, designated T7 promoters. Upstream of the coding sequence for the T7polymerasefusion protein is a minimal promoter (myo-2) preceded by a multiple cloning site (MCS) in which several C. elegans promoters can be inserted. This plasmid (**pGN105** shown in Figure 11) is a basic T7 RNA polymerase plasmid which enables the expression of T7polymerase in C. elegans. Derivatives of this plasmid wherein promoters are cloned into the multiple cloning site, allow for the inducible, constitutive, general and tissue specific expression of T7 RNA polymerase in C. elegans, as expression will be regulated by the promoter cloned in the multiple cloning site.

At page 31-32, please replace the following paragraph:

The T7 RNA polymerase coding sequence was PCR amplified from_λ CE6 using the primers oGN43 (GCCACCGGTGCGAGCTCATGAACACGATTAACATCGC; SEQ ID NO:20) and oGN44 (CACTAGTGGGCCCTTACGCGAACGCGAAGTCCG; SEQ ID NO:21) digested with AgeI/SpeI and inserted in the pGK13 vector digested with AgeI/SpeI. (This vector contains the strong SERCA promoter which drives expression in the pharynx, the vulval muscle, the tail and the body wall muscle). A nuclear localization signal (NLS) of SV40 large T antigen was inserted in front of the T7 polymerase coding sequence by insertion of two overlapping oligo's oGN45 (CCGGATGACTGCTCCAAAGAAGAAGCGTAAGCT; SEQ ID NO:22) and oGN46 (CTCACCGGTAATGAACACGATTAACATCGC; SEQ ID NO:19) into the SacI/AgeI restriction sites. The resulting construct was called **pGN108** as shown in Figure 10. Introduction of this plasmid into C. elegans results in the expression of T7 RNA polymerase in the pharynx, vulva muscle, tail and body wall muscles.

At page 32, please replace the following paragraph:

To test expression and functionality of T7 RNA polymerase in C. elegans under the regulation of the SERCA promoter, pGN108, which encodes the T7RNA polymerase under the control of the SERCA promoter was injected into *C. elegans.* A test vector was coinjected. This

test vector encodes for GFP under the control of a T7 promoter (**pGN401** in Figure 13). The plasmid pGN401 was constructed by inserting two overlapping oligo's oGN41 (CCCGGGATTAATACGACTCACTATA; SEQ ID NO:23) and oGN42 (CCGGTATAGTGAGTCGTATTAATCCCGGGAGCT; SEQ ID NO:24) in the SacI/AgeI opened Fire vector pPD97.82. generating a T7 promoter. Furthermore a selection marker was coinjected to select for transformants (rol6, pRF4). The latter selection vector pRF4 is well known to person skilled in the art. Transgenic F1 could easy be isolated as they display the rol 6 phenotype. These transgenic C. elegans all expressed GFP in the pharynx, the vulval muscle, the tail and the body wall muscle. This data show clearly that theT7 RNA polymerase is functionally expressed under the regulation of the SERCA promoter, and that the expressed T7 RNA polymerase binds to the T7 promoter present in pGN401 and initiates transcription of the GFP gene, which is then functionally expressed, resulting in fluorescence in the muscle tissues where SERCA is inducing the expression of the T7 RNA polymerase.

At page 33-34, please replace the following paragraph:

The Fire vector pPD97.82 was digested with SacI/ AgeI and a T7 promoter sequence was generated by insertion of two overlapping oligo's oGN41 (CCCGGGATTAATACGACTCACTATA; SEQ ID NO:23) and oGN42 (CCGGTATAGTGAGTCGTATTAATCCCGGGAGCT; SEQ ID NO:24) into the SacI/Age/ restriction endonuclease sites. This construct (**pGN400** Figure 12) contains a GFP open reading frame cloned between SacI and EcoRI restriction endonuclease sites under the regulation of the T7 promoter. Any gene, cDNA, or DNA fragment can be cloned in this vector by deleting the GFP gene as a AgeI/ SacI fragment and cloning the DNA fragment of interest into the vector. Preferentially the DNA fragment of interest can be obtained by PCR amplification, inserting the SacI/AfeI sitesin the primers. The resulting DNA fragment after PCR amplification is the digested and the GFP gene in pGN400 is replaced by the amplified DNA fragment. Every vector that contains a T7 promoter could be used for the purpose of T7 RNA polymerase induced expression in C. elegans, such as the commercially available pGEM vectors and the pBluescript vectors. This is clearly shown by the pGN401 vector which expresses GFP under the regulation of the T7 promoter in a transgenic C. elegans which expresses T7 RNA polymerase.

At page 38-39, please replace the following paragraph:

In most two-hybrid experiments a cDNA library is cloned in plasmid pGAD424 (Figure 16) which has been engineered with additional restriction sites in the polylinder such as a Ncol site (Clontech). This library allows for screening of binding proteins in a yeast two hybrid experiment. We constructed a new yeast two hybrid vector with the same possibilities to perform yeast two hybrid, but which contain two additional T7 promoters, so that the vector can be used for T7 RNA polymerase induced pseudo-stable knock-outs. For this we inserted a forward T7 by using a T7 linker (consisting of the following primers aattcttaatacgactcactatagggcc (SEQ ID NO:25) and catgggccctatagtgagtcgtattaag (SEQ ID NO:26)) into the EcoRI-Ncol site of pGAD424. The resulting vector was designated pGAD424-without-FULL-ICE-both-T7. Care was taken to eliminate stop codons and using maximal polylinker compatible amino acids. We adopted the same strategy for the reverse T7 (consisting of both primers atccgtcgacagatctccctatagtgagtcgtattactgca (SEQ ID NO:27) and gtaatacgactcactatagggagatctgtcgacg (SEQ ID NO:28)) with BamH1 and Pstl. To avoid loss of SalI, we included this site in the primer.

At page 39-40, please replace the following paragraph:

An analogous yeast two hybrid vector was constructed based on pAS2 (Clontech). By partial EcoRV digestion we were able to remove a significant part of the cyh2 gene. The right construct can be isolated and checked by a restriction digest with BglII. this restriction site is present in the EcoRV fragment of PAS2 to be eliminated. This elimates the cyh2 gene which is slightly toxic gene and involved in growth retardation. This gene is non-essential for the performing of RNAi and Yeast two hybrid experiments. After the elimination of the EcoRV fragment, The EcoRI restriction site which is located between the DNA sequence encoding for GAL4DB and HA (epitope) becomes unique for the plasmid, and can be used to subsitute HA with a T7 promoter containing linker. This ensures persistence of all restriction sites, allowing both in frame cloning and compatibility with previous vectors and pGAD424. We used the following linker (primers: aattcttaatacgactcactatagggca (SEQ ID NO:25) and tatgccctatagtgagtcgtattaag (SEQ ID NO:29)) using EcorI and Ndel cloning sites. we adopted the same strategy for the reverse T7 (primers: gatccgtcgacagatctccctatagtgagtcgtattactgca (SEQ ID NO:27) catgggccctatagtgagtcgtattaag (SEQ ID NO:26) and gtaatacgactcactatagggagatctgtcgacg

(SEQ ID NO:28)) with BamH1 and Pstl. To avoid loss of Sall we included it in the primer.The resulting vector was designated pAS2-cyh2HA+both T7-final.